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ULTRASTRUCTURAL LOCALIZATION OF CARBOHYDRATE DETERMINANTS OF LIPOPOLYSACCHARIDE OF COXIELLA BURNETII (PHASE I) BY MONOCLONAL ANTIBODIES

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Coxiella burnetii, the etiological agent of Q fever, is an obligate phagolysosomal bacterium and an unorthodox spore former. Morphologically distinct cell types, produced by a developmental cycle, are defined as large cell (LCV) and small cell variants (SCV).[1,2] Recent study has shown phase I lipopolysaccharide (LPS-I) is differentially expressed among the cell variants.[3] This antigenic variation was investigated further by use of a series of monoclonal antibodies (MAbs), directed against LPS-I,[4] and post-embedding immunolabelling techniques. Since these MAbs recognize carbohydrate determinants of LPS-I,[4] the specificity of the labelling was tested by carbohydrate reducing and oxidizing reagents prior to labelling.

C. burnetii (9MIC7 strain), which was purified from infected yolk sac material of hen eggs by Renografin gradient centrifugation, was fixed for 3 h in 1.5% glutaraldehyde and 0.2% picric acid in 66mM Na-cacodylate buffer, pH = 6.8; pre-embedded in 2% Difco Nobel agar; and then rinsed once (15 min) in the same buffer. Dehydration was first carried out in 50% methanol (15 min), then in 70% (two changes, 1 h each); followed by an intermediate step (66% L R White resin in 70% methanol for 30 min). Polymerization was carried out in L R White at 50°C for 24 h. Immunolabelling was performed on 60-100 nm thin sections, on nickel grids, using three different MAbs (#1135, #2011, #1363) against LPS-I as 1° antibody, and goat anti-mouse IgM colloidal gold (5 nm) (Janssen) as 2° antibody. The specificity of MAbs for carbohydrate determinants of LPS-I was tested by subjecting the sections to either aq. Na-m-periodate (5 mM) for 1, 2, 4, 6 h or water saturated with phenol for 3 min at 68°C before labelling. The sections were then stained with 0.5% uranyl acetate in 50% ethanol (30 sec) and lead citrate (30 sec) and examined with a Joel 100B TEM operated at 80 kv.

All three MAbs revealed abundant epitopes of LPS-I on the cell wall of the SCV (Fig. 1). Fewer or no LPS-I epitopes were found on the LCV (Fig. 1). Most of the gold labelling was obtained with MAb #1135 (Fig. 1). The gold label was mostly confined to the periplasmic space and the exterior of the SCVs (Fig. 2). By incubating the sections in Na-m-periodate, an efficient oxidising agent for carbohydrate, prior to labelling with MAb #1135, the quantity of labelling with the gold probe was reduced. The intensity of gold labelling was reduced after 1 h (Fig. 3) and eliminated after 6 h incubation (Fig. 4). Gold labelling was also absent in phenol-water treated sections.

We conclude that the LPS-I epitope recognized by MAb #1135 is differentially expressed among the cell variants. The pattern of immunolabelling suggests that the morphologic variants undergo differentiation as a genetic mechanism for antigenic masking. The specificity of the MAbs for the carbohydrate epitopes of the LPS was verified by the use of either Na-m-periodate or phenol-water. The absence of the label after chemical treatment stresses caution in the use of etching solutions, which have been described for enhancing antigen recognition.[5]

References

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FIG. 1.--Thin-section of C. burnetii embedded in L R White resin. Immunolabelling of LPS-I on cell wall of SCV (arrows). Note absence of labelling on LCV (*). Bar = 250 nm.
FIG. 2.--Higher magnification of SCV showing labelling of outer membrane. Bar = 50 nm.
FIG. 3.--Section of SCVs treated with Na-m-periodate (1 h) before labelling. Note reduction of labelling. Bar = 200 nm.
FIG. 4.--Treatment with Na-m-periodate (6 h). Near absence of labelling of LPS-I. Bar = 250 nm.